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Particle Size Reduction Optimization of *Laminaria* spp. Biomass for Enhanced Methane Production

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Abstract

Recent studies have reported improved biogas and methane yield from marine biomass when the particle size is mechanically reduced and the specific surface area available to enzymes is increased prior to anaerobic incubation. Although the advantage of reducing the particle size has been identified, an ideal particle size that would offer greater yield with a positive energy balance has not been identified for such substrate to date. As particle size reduction by mechanical means is often highly demanding in energy, this paper attempts to fill this gap for macroalgal biomass by identifying the particle size distribution allowing the highest biogas and methane yields obtained in a previous work. The study estimated that when about 80% of the particles are sized below 1.6 mm², a biogas and methane yield improvement of up to 52% and 53% respectively can be achieved. The results are discussed in relation to the biogas yield, related methane content and potential inhibitory phenomena occurred during the fermentation.

Keywords: Methane; Biogas; Macroalgae; Seaweeds, Mechanical Pre-treatment; Particle Size.

Abbreviations: FA, Free Ammonia; SR Schopper-Riegler number; VFA, Volatile Fatty Acids; VS, Volatile Solids.

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1 Introduction

Biomass research and developments have been presented in many papers and editorials [1-4]. The mechanical pre-treatment step constitutes a necessary phase to treat any type of biomass prior to any further pre-treatment or anaerobic digestion [5]. A variety of mechanical pretreatments has been developed with the scope of reducing the particle size of the biomass and therefore increasing the specific surface area available for bacterial action [6]. As mentioned in the literature, the particle size is a very important factor positively affecting the biogas production from any type of biomass. A very recent review reports that the particle size reduction is directly correlated with increased biomethanation from lignocellulosic substrates, especially at thermophilic conditions [7]. Such positive effect has been confirmed on very flexible plant biomass such as grass for both biogas [8] and biomethane [9] production. Currently, very comprehensive literature reviews correlating substrates [10], particle size and energy consumption [11] are available for further research and development. However, despite the intensive research of the field, the particle size reduction has often consistently incurred high bioconversion costs, like in the case of lignocellulosic biofuels. Moreover, excessive particle size reduction does not always incur greater yields for all substrates [12]. In this regard, Deublein and Steinhauser [13] claim the relationship between the increased surface area and the increased biogas yield is not linear, as the contribution of very fine particles is lower than that of the bigger ones. This observation is crucial for a critical assessment of the most appropriate particle size to target for a particular substrate, as obtaining finer particles makes the pretreatment more expensive, and may result in negligible yield increases. Seaweeds' bioenergy potential has been identified almost three decades ago [14], and it is now being regarded as the biomass resource of the future generations.

This work constitutes a continuation of a previous project [15], in which *Laminaria* spp. biomass were mechanically comminuted by a Hollander beater varying the machine's settings along with the temperature of fermentation in the reactors. The derived biogas and methane yields were used as the responses for a complex system optimisation in order to identify the optimal system input variables by using the response surface methodology (RSM). The highest biogas yield achieved was 685 mL gTS⁻¹, of which 430 mL gTS⁻¹ was CH₄. In this study, the particle size distribution and the pulp drainability of the feedstock resulting from the proposed pretreatment of [15] are here reported and discussed in relation to the biogas and methane yield. The analyses are performed with the goal of characterizing the most appropriate biogas-producing pulp, referring to the machine's settings used in the previous work, which are provided in Table 1.

2 Materials and Mechanical Pre-treatment

Laminaria spp. biomass was collected in Howth, Dublin (Ireland) in June 2013 and used immediately, while the sludge used as inoculum was collected in the waste water treatment plant of Celtic Anglian water Ltd., Ringsend, Dublin (Ireland) and used the same day of the fermentation experiments. The characterization of all the material involved in the experiment is reported in [15], while sludge's characterization is illustrated in Table 2. The equipment used for the particle size reduction consists of a modified Hollander beater, model Reina fully described in previous publications [15, 16]. A section view of the machine is shown in Figure 1 to provide continuity and allow visualizing one of the study variables, i.e. the machine's gap (MG).

3 Experimental Methodologies

The biomass particle size described in the literature for macroalgae [17-19] has been mostly obtained by sieving the treated substrate through a screen of known dimensions, and the particle size is then referred to as mean particle diameter (spherical or squared particles assumed). Particle size analysis revealed this assumption is incorrect in the case of macroalgae's pretreatment with the Hollander beater, as the resulting particle is extremely irregular in both shape and dimensions. Therefore, in this work the particle size analysis uses the metric of the effective surface area, detected in mm^2 , and will be hereon referred as maximum frontal surface. The total amount of the different types of macroalgal pulp is 9 and can be achieved varying the machine's gap and the treatment time, as it can be extrapolated by Table 1.

3.1 Particle size distribution evaluation

The maximum frontal surface and distribution of the pulp's particles produced by treating the biomass with the Hollander beater are unique to this specific machine. It was decided to use the superficial surface area in mm^2 identified by the means of image processing analysis.

Since the particles will be inspected with a camera, the treated solution samples need to be diluted in order to obtain an acceptable distance between particles. Most samples required a 2:1 dilution with tap water, for more concentrated samples however the dilution was further increased to 6:1. A summary of the dilutions used in this research with respect to the other settings can be seen in Table 2, where the label ID identifies each sample, i.e. G represents the gap setting and T the treatment duration. In order to express the particle distributions obtained at different machine settings in a comparable way, these dilutions will be taken into account in order to normalize the results appropriately.

The samples are divided into four 100 mL batches before they are inspected. Each batch is then poured into a glass tray with a white base where the particles are coaxed apart as much as possible. Various images of the resulting particle spread are then taken, making sure the entire tray is captured. Pictures of the samples were taken using a Canon Powershot G9 camera at high resolution and from the same distance with the aid of a tripod. The camera's specifications and settings are regulated as follows: digital zoom = 1; compression = superfine; F-stop = 3.5; exposure time = 1/600 sec; ISO speed = ISO-100; white balance = manual; flash = none; focal length = 22 mm; max aperture = 3.625. The pictures were then processed using the image processing software ImageJ, which converts each of them into a binary image for easier characterization, as can be seen in Figure 2. ImageJ is able to calculate the area of all individual particles and their quantities. Both these measurements are used to build a statistical size distribution with Microsoft Excel (2010).

3.2 Substrate's drainability assessment

Pulp's drainability constitutes a very useful tool to associate pulped biomass with eventual digestate's dewaterability issues that arise when scaling up the process in industrial facilities [20]. Above all, the drainability test is fundamental to directly and univocally identify the Hollander beater's operational parameters effects on the particle size reduction. In order to fulfil this purpose, this research employs a Schopper-Riegler apparatus (Figure 3). The Schopper-Riegler method is used to define the degree of beating in the paper industry (for which the Hollander beater is designed), and so it constitutes a valid tool to test the macroalgal pulp's particle suspension in water obtained in this research.

The procedure that is followed for the Schopper-Riegler method is the ISO 5267-1:1999 (E) [21]. For each of the 9 samples, a specific Schopper-Riegler number (SR) is identified. The higher the SR, the more drainable the pulp is.

4 Results and Discussion

The particle frontal surface distribution and the pulp drainability of the feedstock resulting from the Hollander beater pretreatment are reported and discussed in relation to the biogas and methane yield.

4.1 Particle size distribution effect on methane formation

The operational parameters, the resulting biogas yields and its methane content percentages are reported in Table 4, which immediately reveals that samples made of particles with smaller frontal surface (G0T5, G0T10, and G0T15) produce biogas with the highest methane content at 40°C and 50°C, confirming the beneficial effects of the treatment on methane content during biogas production. The particle analysis results arising from the treated samples shown in Table 4 are then plotted for each ID code which represents the parameters setting, i.e. the ID G0T5 is the sample treated at gap MG=0 and BT=5 minutes. The data obtained is provided in Table 5 and presented in Figure 4. The X axis is presented in logarithmic format to enhance clarity.

Figure 4 reveals that samples G0T15 and G0T10 have a considerably higher amount of particles with small frontal surface compared to the rest. According to the biogas and methane results obtained in Table 4, the worst performing sample (G10T5) proved in the particle size distribution plots to also contain the fewest number of particles. Moreover, samples G5T5 to

G10T5 can be seen in Table 5 to contain less particles in total, but a greater number of particles in the higher ranges of area.

The total solids concentration in every sample can be considered to be the same as in [15], and the yields comparison in Table 4 was made on the same dry matter basis. This means that the only difference between the samples is the available surface area for enzymatic degradation during the hydrolysis step, which increases with the amount of particles in the sample. For any given volume of material, if it consists of a larger number of smaller particles, it will have a greater overall surface area than a small number of larger particles. The results above are in line with the benefits of the comminution concept reported in literature. It was expected that the highest methane-producing sample achieved in study D would have been G0T15 at 50°C, instead the best performing sample was G0T10 at 50°C which this study found to not have the highest number of particles out of the tested samples. Therefore an excessive reduction in particle size is reached with the proposed pretreatment at settings G0T15 (MG=0, BT=15 minutes), leading to an inhibition effect.

As mentioned in the literature [22-24], if the particle size is below a critical value, the rate of hydrolysis is much greater and therefore a buildup of VFAs may occur in the reactor especially at the beginning of the AD as the biodegradation is more concentrated around the hydrolysis reactions, leading to a drastic drop in pH [25]. This in turn would have an inhibitory effect on the methanogenic bacteria, and therefore reducing the amount of biogas and methane produced as pH values below 6.5 have a toxic effect on methanogens [26], making the hydrolysis the rate limiting step in the anaerobic digestion process. A buildup of VFAs may occur also when digesting at thermophilic conditions [27], as a higher temperature can result in higher FA and so in an increased toxicity level and lower process stability, as found in another study on Irish *Laminaria* spp. digestion [28], where the authors ground the

biomass to less than 1 mm, and found both biogas and methane productions from mesophilic and thermophilic digestions to be inhibited as pH levels dropped below 6.5. Drops in pH when digesting *Laminaria* spp. were also identified by [29, 30], after excessively reducing the particle sizes of the feedstock. In this research however, the pH readings carried out across the digestion period did not indicate a consistent decrease in pH and thus experiencing no inhibition related to excessive VFAs accumulation, despite entering into the thermophilic range and having particle reduction comparable to those studies that experienced inhibition. This lack of pH inhibition is also reported in [17, 20, 31]. Thus the reason of G0T10's better performance compared with G0T15 cannot be attributed to an excessive accumulation of VFAs. The interaction between NH_3 , VFAs and pH determines an "inhibited steady state" [32], where the process is running stably but with a lower methane yield [33]. Ammonia is produced by the biological degradation of the nitrogenous matter. Macroalgal composition usually present high nitrogen content, and consequent ammonia-N inhibition, particularly in thermophilic conditions as highlighted by Chen et al. [32]. High content of nitrogen may also lead to COD/N imbalance, and consequently to inhibition of methanogenesis [34]. The cause of this cannot be fully answered here by the measurements taken and this aspect should be further investigated via analysis of free ammonia NH_3 (FA) and VFAs concentration across the whole AD period. Furthermore, recorded values of H_2S were found above 100 ppm in all co-digesting samples. H_2S can be regarded as the toxic form of sulfide since it can diffuse into the cell membrane [35]. Therefore, concentration of other inhibitors, such as sulphide, should also be examined to properly identify the reason of such behaviour.

In general, it is proposed that a blend of large and small particles may in fact be the ideal substrate for high biogas yields. The smaller particles would enable an initial rapid rate of

hydrolysis, which in turn would build up the levels of nutrients suitable for methanogenic bacteria to begin producing biogas. Once these smaller particles are consumed however, the larger particles would provide a reduced surface area, and therefore regulate the subsequent levels of hydrolysis in such a way that all the reactions involved in the digestion are stable and balanced. These requirements seem to be fulfilled by sample G0T10 which allows the highest yields of biogas and methane production. In this sample it was found that the smallest particle size range percentages are very similar (about 20% each), and more than 80% of G0T10's macroalgal particles have a specific surface area below the ideal particle size of 1–2 mm identified by Shell for lignocelluloses [36]. Moreover, the worst performers of Table 4, namely G10T5, G5T10 and G10T15 have a proportion of approximately 50% of particles below 1 mm². Thus for optimum yields this proportion should be increased to about 80%. This represents a very important finding, as it appears that seaweed conversion to methane would greatly benefit from a pretreatment capable of achieving targeted particle size ranges. The results show the optimum range to be below a frontal surface of 1.6 mm², however it is not possible to identify a unique value of an ideal particle's surface area for the Hollander beater. For this reason a drainability study is needed to provide an additional and more accurate metric to characterize the *Laminaria* spp. pulp obtained by this pretreatment.

4.2 Drainability characterization results and discussion

In the fertilizer upgrading process, the digestate needs to be drained of water to reduce its net weight and thus improve the logistics of applying the fertilizer to agricultural land.

Drainability is an extremely important property that facilitates the separation of liquid digestate from its most solid parts and thus reduces the costs of the fertilizer upgrading step [20]. Results of the drainability test are expressed in terms of SR number in Table 6. The SR number has an inversely proportional relationship with drainability, meaning that the biomass

samples subjected to longer beating times have lesser drainability. Table 6 reveals that the pulp obtained at MG=0 is the least drainable. Pulps treated for 5 minutes are not consistently affected by MG variations (between 78 and 80 SR), while for longer treatment times MG plays a fundamental role in affecting the drainability of the macroalgal pulp. Keeping BT fixed at 10 minutes, reducing MG from 10 to 5 and from 5 to 0, will both cause a decrease in dewaterability of around 10%; while keeping BT fixed at 15 minutes and reducing MG from 10 to 5 and from 5 to 0, will cause a decrease in dewaterability of 13% on average. A mean decrease of 17.4% in dewaterability can be observed between coarser pulp (MG=10 with a mean SR of 73) and ideal pulp (SR=88). Comparison with the following peer-reviewed studies shows the effect of a pretreatment on the substrate's dewaterability is strictly associated to its nature and morphology. For instance, Xu et al. [37] studied ultrasonic pretreatment effect on sludge before digestion, and found it has a detrimental effect on dewaterability which resulted in a decrease of about 98% compared to unsonicated sludge. Drainability values obtained with the Hollander beater in this research show that macroalgal pulp is more than 80% more dewaterable than sludge after ultrasonic treatment. However, in another study [38], particle size reduction was found to be instead positively correlated with dewaterability of food waste digestate, with an improvement of about 12.5% (time-to-filter) compared to coarse particles. In this case, the grinding effect (from 8 to 2.5 mm) incurred extra specific CH₄ yield of 9–34%, while for macroalgal pulp used in this research values up to 53% were achieved.

Anaerobic digestion in general has also been found to be beneficial at enhancing the drainability performance of the digestate, particularly when using thermophilic ranges [39]. However, the investigation conducted by Suhartinia et al. on sugar beet pulp digestate shows mesophilic digestion results in poor dewaterability. The authors suggest that the water is

strongly bound to the digestate possibly due to either colloidal solids or high molecular weight extracellular polymers blinding the filter pores. It is believed this can also be the cause of reduced drainability after treatment with the Hollander beater.

5 Conclusions

During the course of this study, the following findings were made:

- The gap size setting of the beater and beating time have a significant effect on the nature of the particles produced by the beater. The beater produces large, irregularly shaped particles at wider gap sizes and shorter beating times, and small particles at narrower gap sizes with longer beating times.
- The highest biogas yields involved particles which were smaller and greater in number. These samples also had a particle surface distribution which was more uniform, namely 80% below 1.6 mm² of frontal surface, which represents the recommended threshold of particle size reduction for *Laminaria* spp. seaweeds.
- Low yields were obtained from material, in which the particles were less in number and relatively large. This is probably due to there being a reduced surface area available for enzymatic degradation.
- It was found that a narrower gap size (MG=0) and a longer beating time should be used when pre-treating seaweed biomass with the Hollander beater, but at the same time excessive beating times lead to a reduced biogas yield, as do short beating times with wider gap sizes. The reason is not yet clear and cannot be attributed to an excessive accumulation of VFAs. This aspect should be further investigated via analysis of free ammonia (FA), sulfide and VFAs concentration across the whole AD period.

The smaller the gap MG used for the macroalgae's comminution, the lower is the drainability of the pulp. A high SR value of 88 characterizes the ideal macroalgal pulp for maximized biogas and methane production. Reduced dewaterability of the substrate can affect the cost of digestate upgrading to soil fertilizer, and must be taken into consideration when scaling up the pretreatment process.

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Table Captions:

Table 1. Process variables and values.

Table 2. Sludge characterizations used to determine the sludge contribution (Celtic Anglian Water Ltd.).

Table 3. Operational parameters, dilution factors and sample ID.

Table 4. Variables, sample ID and correlated biogas yield.

Table 5. Particle distribution for equalized volumes of samples.

Table 6. Drainability values obtained by Schopper-Riegler method.

Fig. Captions:

Fig. 1. Cross sectional view of a Hollander beater (Source: woodwardesigns.com)

Fig. 2. Examples of fine (a) and coarse (b) particle image and their binary equivalents (c, d).

Fig. 3. Schopper-Riegler apparatus.

Fig. 4. Particle size distribution plot for the 9 samples.

Table 1. Process variables and values

Variables	Values		
Pre-treatment time (minutes)	5	10	15
Machine chopping gap (turns)	0	5	10
Incubation temperature (°C)	30	40	50

Table 2. Sludge characterizations used to determine the sludge contribution (Celtic Anglian Water Ltd.)

Parameters	Value
Total Solids (TS) [%]	4.0±0.04
Volatile Solids (VS) [%]	69±1.2
COD [mg/l]	68.0±2.6
Ammonia [mg/l]	2.04±0.01
Alkalinity [mg/l]	10.6±0.40
VFA's [mg/l]	342±22

Table 3. Operational parameters, dilution factors and sample ID.

Machine's Gap (MG) [turns]	Beating Time (BT) [minutes]	ID	Dilution degree
10	5	G10T5	2:1
	10	G10T10	2:1
	15	G10T15	2:1
5	5	G5T5	2:1
	10	G5T10	2:1
	15	G5T15	6:1
0	5	G0T5	2:1
	10	G0T10	6:1
	15	G0T15	6:1

Table 4. Variables, sample ID and correlated yields.

MG [turns]	BT [minutes]	T [°C]	ID	Averaged biogas yield [mL gTS⁻¹]	CH₄ [*] [%]
0	5	40	G0T5	207.6	47
	10	30; 50	G0T10	675.1;651.0	40;48
	15	40	G0T15	320.4	48
5	5	30; 50	G5T5	503.6;203.6	43;37
	10	40	G5T10	166.3	44
	15	30; 50	G5T15	476.8;255.2	44;41
10	5	40	G10T5	138.2	42
	10	30; 50	G10T10	527.1;335.5	44;42
	15	40	G10T15	190.8	47

^{*}*From [15] including the inoculum's contribution*

Table 5. Particle distribution for equalized volumes of samples.

Size range (mm²)	Number of particles counted (including dilution factor)								
	G0T 15	G0T10	G0T5	G5T15	G5T10	G5T5	G10T15	G10T10	G10T5
0.1-0.2	1199	1130	108	295	121	84	81	58	38
0.2-0.4	1453	1372	121	382	149	106	96	70	49
0.4-0.8	1472	1358	118	480	180	126	105	73	61
0.8-1.6	1208	1091	105	500	175	125	97	73	58
1.6-3.2	695	664	84	403	149	108	81	62	56
3.2-6.4	272	321	56	273	109	91	58	43	40
6.4-12.8	83	95	31	143	67	60	44	32	24
12.8-25.6	15	18	8	37	25	35	28	19	10
25.6-51.2	2	1	2	6	6	16	16	10	12
51.2-102.4	0	0	0	0	1	4	3	2	4
102.4- 204.8	0	0	0	0	0	0	1	1	4
204.8- 409.6	0	0	0	0	0	0	0	1	1
Total particles	6399	6050	633	2519	982	755	610	444	357

Table 6. Drainability values obtained by Schopper-Riegler method.

MG [turns]	BT [minutes]	SR	Corresponding Volume [mL]
10	5	79	210
	10	71	285
	15	68	315
5	5	78	218
	10	80	200
	15	78	218
0	5	80	220
	10	88	120
	15	92	75

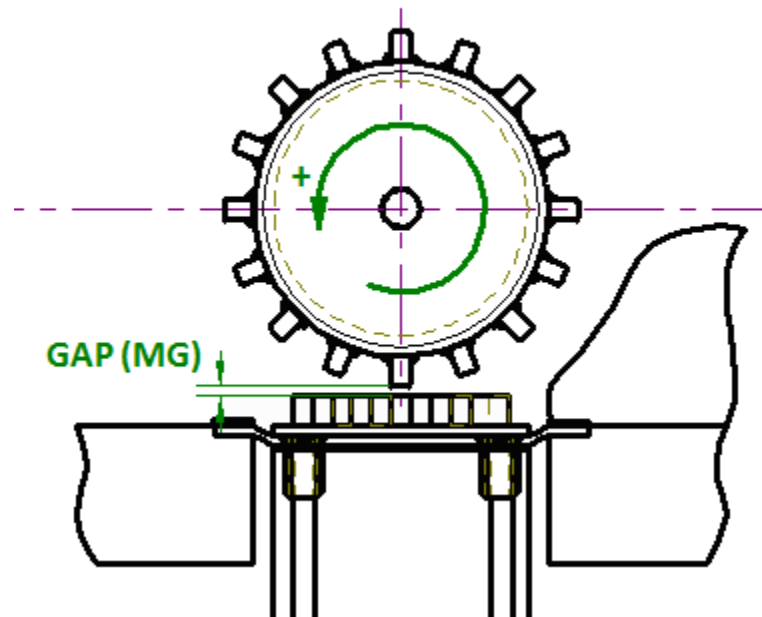


Fig. 1 Cross sectional view of a Hollander beater (Source: woodwardesigns.com)

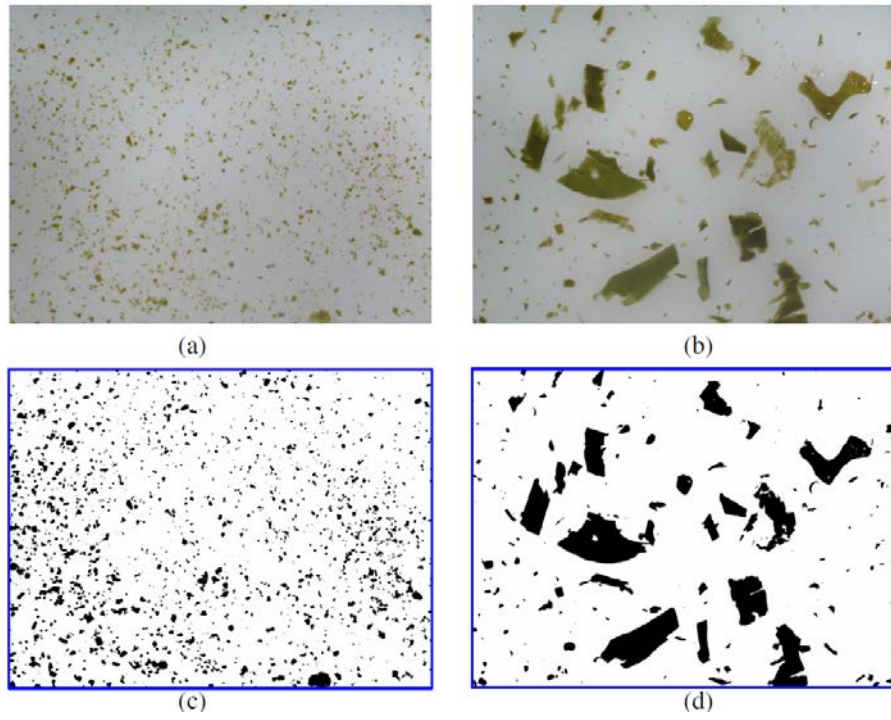


Fig. 2 Examples of fine (a) and coarse (b) particle image and their binary equivalents (c, d).



Fig. 3 Schopper-Riegler apparatus.

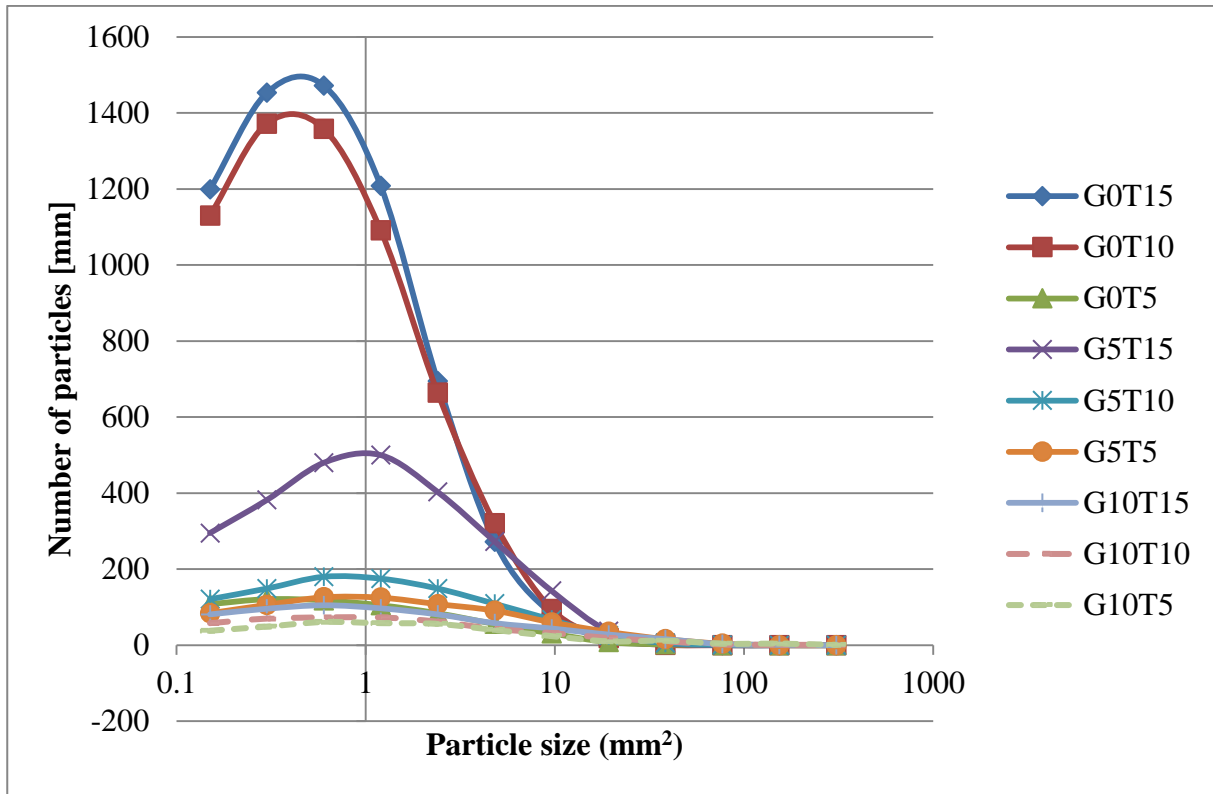


Fig. 4 Particle size distribution plot for the 9 samples.